

CLAIMS

WHAT IS CLAIMED IS:

1 1. An isolated nucleic acid comprising a nucleotide sequence
2 encoding at least about five contiguous amino acids of an ESX transcription factor
3 variable region polypeptide, wherein said variable region has an amino acid
4 sequence as set forth in SEQ ID NO: 7 or conservative substitutions of said amino
5 acid sequence.

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A 1 2. The isolated nucleic acid of claim 1, wherein said nucleic acid
2 encodes an ESX transcription factor having an amino acid sequence as set forth in
3 ~~SEQ ID NO: 3~~
4 ~~SEQ ID NO: 3~~.

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A 1 3. The isolated nucleic acid of claim 2, wherein said nucleic acid
2 has a nucleotide sequence as set forth in ~~SEQ ID NO: 1~~
3 ~~SEQ ID NO: 1~~.

A 1 4. The nucleic acid of claim 1, wherein said nucleic acid is
2 amplified from a genomic library using the primer pairs designated by ~~SEQ ID NO:~~
3 ~~13 and SEQ ID NO: 14~~
4 ~~13 and SEQ ID NO: 14~~.

1 5. The nucleic acid of claim 1, wherein said nucleic acid hybridizes
2 to a clone of a human ESX gene under stringent conditions.

1 6. The nucleic acid of claim 1, wherein said nucleic acid further
2 comprises a vector.

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A 1 7. The nucleic acid of claim 1, wherein said variable region has an
2 amino acid sequence as set forth in ~~SEQ ID NO: 7~~
3 ~~SEQ ID NO: 7~~.

A 1 8. The isolated nucleic acid of claim 1, wherein said nucleotide
2 sequence has a smallest sum probability of less than about 0.5 when compared to a
3 nucleotide sequence as set forth in ~~SEQ ID NO: 6~~
4 ~~SEQ ID NO: 6~~ using a BLASTN algorithm
using default parameters.

1 9. The isolated nucleic acid of claim 8, wherein said smallest sum
2 probability is less than about 0.2.

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A 1 10. An isolated nucleic acid comprising a label and a nucleotide
2 sequence encoding a carboxy terminal domain of an ESX transcription factor,

3 wherein said carboxy terminal domain has an amino acid sequence as set forth in
4 SEQ ID NO: 12 or conservative substitutions of said amino acid sequence.

1 11. The nucleic acid of claim 10, wherein said nucleic acid is free
2 of dideoxynucleotides.

1 12. The nucleic acid of claim 10, wherein said nucleic acid is single
2 stranded.

1 13. The nucleic acid of claim 12, wherein said nucleic acid is a
2 sense strand.

1 14. The isolated nucleic acid of claim 10, wherein said label is a
2 radionuclide.

1 15. An isolated nucleic acid encoding a human ESX transcription
2 factor polypeptide comprising at least 8 contiguous amino acids from a polypeptide
3 sequence encoded by a nucleic acid as set forth in ~~SEQ ID NO: 1~~, wherein:
4

5 said polypeptide, when presented as an antigen, elicits the
6 production of an antibody that specifically binds to a polypeptide sequence
7 encoded by a nucleic acid as set forth in ~~SEQ ID NO: 1~~; and
8

9 said polypeptide does not bind to antisera raised against a
10 polypeptide encoded by a nucleic acid sequence as set forth in SEQ ID NO: 1, that
11 has been fully immunosorbed with a polypeptide encoded by a nucleic acid
12 sequence as set forth in ~~SEQ ID NO: 1~~.

1 16. An isolated nucleic acid comprising a nucleotide sequence
2 encoding at least about ten contiguous amino acids of a murine ESX transcription
3 factor polypeptide having an amino acid sequence as set forth as mESX in Figure 5
4 or conservative substitutions of said amino acid sequence.

1 17. The nucleic acid of claim 16, wherein said nucleic acid encodes
2 an ESX transcription factor having an amino acid sequence as set forth as mESX in
3 Figure 5.

1 18. The nucleic acid of claim 17, wherein said nucleic acid has a
2 nucleotide sequence as set forth in ~~SEQ ID NO: 15~~.
3

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1 19. The nucleic acid of claim 16, wherein said nucleic acid is
A 2 amplified from a genomic library using the primer pairs designated by SEQ ID NO:
A 3 ~~16 and SEQ ID NO: 17.~~ ~~16 and SEQ ID NO: 17.~~

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1 20. The nucleic acid of claim 16, wherein said nucleic acid
2 hybridizes to a clone of a murine ESX gene under stringent conditions.

1 21. The nucleic acid of claim 16, wherein said nucleic acid further
2 comprises a vector.

1 22. The nucleic acid of claim 16, wherein said nucleic acid is
2 labeled.

1 23. The nucleic acid of claim 22, wherein said nucleic acid is free
2 of dideoxynucleotides.

1 24. The nucleic acid of claim 22, wherein said nucleic acid is single
2 stranded.

1 25. The nucleic acid of claim 24, wherein said nucleic acid is a
2 sense strand.

1 26. The isolated nucleic acid of claim 22, wherein said label is a
2 radionuclide.

1 27. An isolated nucleic acid encoding a murine ESX transcription
2 factor polypeptide comprising at least 8 contiguous amino acids from a polypeptide
3 shown as MESX in Figure 5, wherein:

4 said polypeptide, when presented as an antigen, elicits the
5 production of an antibody that specifically binds to the polypeptide designated
6 mESX in Figure 5; and

7 said polypeptide does not bind to antisera raised against the
8 polypeptide designated mESX in Figure 5, that has been fully immunosorbed with
9 the polypeptide designated mESX in Figure 5.

1 28. An isolated human ESX polypeptide, said polypeptide
2 comprising a subsequence of at least 5 contiguous amino acids of a polypeptide
3 encoded by a nucleic acid selected from the group consisting of SEQ ID NO:4,

4 SEQ ID NO:6, SEQ ID NO:9, and SEQ ID NO:11, or conservative substitutions of
5 said polypeptide subsequence.

1 29. The polypeptide of claim 28, wherein said polypeptide
2 comprises a subsequence of at least 50 contiguous amino acids encoded by a
3 nucleic acid selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6,
4 SEQ ID NO:9, and SEQ ID NO:11, or conservative substitutions of said
5 polypeptide subsequence.

1 30. The polypeptide of claim 29, wherein said polypeptide is a
2 polypeptide encoded by a nucleic acid selected from the group consisting of SEQ
3 ID NO:4, SEQ ID NO:6, SEQ ID NO:9, and SEQ ID NO:11.

1 31. An isolated human ESX polypeptide, said polypeptide
2 comprising at least 8 contiguous amino acids from a polypeptide sequence encoded
3 by a nucleic acid as set forth in ~~SEQ ID NO: 1~~, wherein:

4 said polypeptide, when presented as an antigen, elicits the
5 production of an antibody which specifically binds to a polypeptide encoded by a
6 nucleic acid as set forth in ~~SEQ ID NO: 1~~; and

7 said polypeptide does not bind to antisera raised against a
8 polypeptide encoded by a nucleic acid sequence as set forth in SEQ ID NO: 1
9 which has been fully immunosorbed with a polypeptide encoded by a nucleic acid
10 sequence as set forth in ~~SEQ ID NO: 1~~

1 32. The isolated polypeptide of claim 31, wherein said polypeptide
2 is encoded by a nucleic acid as set forth in ~~SEQ ID NO: 1~~.

1 33. An isolated murine ESX polypeptide, said polypeptide
2 comprising a subsequence of at least 10 contiguous amino acids of the polypeptide
3 designated mESX in Figure 5, or conservative substitutions of said subsequence.

1 34. The polypeptide of claim 33, wherein said polypeptide
2 comprises a subsequence of at least 50 contiguous amino acids of the polypeptide
3 designated mESX in Figure 5, or conservative substitutions of said polypeptide
4 subsequence.

1 35. The polypeptide of claim 33, wherein said polypeptide is a
2 polypeptide having the sequence of the polypeptide designated mESX in Figure 5.

1 36. An isolated murine ESX polypeptide, said polypeptide
2 comprising at least 8 contiguous amino acids from the polypeptide sequence
3 designated mESX in Figure 5, wherein:

4 said polypeptide, when presented as an antigen, elicits the
5 production of an antibody which specifically binds to a polypeptide having the
6 sequence designated mESX in Figure 5; and

7 said polypeptide does not bind to antisera raised against a
8 polypeptide having the sequence designated mESX in Figure 5 which has been
9 fully immunosorbed with a polypeptide having the sequence designated mESX in
10 Figure 5.

1 37. The polypeptide of claim 36, wherein said polypeptide has the
2 amino acid sequence designated mESX in Figure 5.

1 38. An anti-human ESX antibody which specifically binds to a
2 polypeptide comprising at least 10 contiguous amino acids from a polypeptide
3 encoded by a nucleic acid as set forth in ~~SEQ ID NO: 1~~, wherein:

4 said polypeptide, when presented as an antigen, elicits the
5 production of an antibody which specifically binds to a polypeptide encoded by a
6 nucleic acid as set forth in ~~SEQ ID NO: 1~~; and

7 said polypeptide does not bind to antisera raised against a
8 polypeptide encoded by a nucleic acid sequence as set forth in SEQ ID NO: 1
9 which has been fully immunosorbed with a polypeptide encoded by a nucleic acid
10 sequence as set forth in ~~SEQ ID NO: 1~~.

1 39. The antibody of claim 38, wherein said polypeptide comprises
2 16 contiguous amino acids are encoded by a nucleotide sequence as set forth in
3 SEQ ID NO: 11.

1 40. The antibody of claim 38, wherein said antibody is monoclonal
2 antibody.

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1 41. A recombinant cell expressing the anti-human ESX antibody of
2 claim 38.

1 42. An anti-human ESX antibody which specifically binds to a
2 polypeptide comprising at least 10 contiguous amino acids from a polypeptide
A 3 encoded by a nucleic acid as set forth in ^{SEQ ID NO:1}~~SEQ ID NO:1~~, wherein:
4 said polypeptide, when presented as an antigen, elicits the
5 production of an antibody which specifically binds to a polypeptide encoded by a
A 6 nucleic acid as set forth in ^{SEQ ID NO:1}~~SEQ ID NO:1~~; and
7 said polypeptide does not bind to antisera raised against a
8 polypeptide encoded by a nucleic acid sequence as set forth in SEQ ID NO: 1
9 which has been fully immunosorbed with a polypeptide encoded by a nucleic acid
A 10 sequence as set forth in ^{SEQ ID NO:1}~~SEQ ID NO:1~~.

1 43. The antibody of claim 42, wherein said polypeptide comprises
2 16 contiguous amino acids are encoded by a nucleotide sequence as set forth in
3 SEQ ID NO:11.

1 44. The antibody of claim 42, wherein said antibody is monoclonal
2 antibody.

1 45. A recombinant cell expressing the anti-human ESX antibody of
2 claim 42.

1 46. A method of detecting dysregulation of an ESX gene in an
2 organism, said method comprising the steps of
3 i) providing a biological sample of said organism; and
4 ii) determining whether an ESX gene in said sample is
5 expressed at a higher level or is present at a greater copy number compared to an
6 ESX gene in a corresponding tissue known to be healthy.

1 47. The method of claim 46, wherein said dysregulation is a result
2 of ESX gene amplification in cells of said sample.

1 48. The method of claim 47, wherein said gene amplification is
2 detected by comparative genomic hybridization or FISH.

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1 49. The method of claim 46, wherein said dysregulation is a result
2 of ESX gene rearrangement in cells of said sample.

1 50. The method of claim 46, wherein expression of said ESX gene
2 at a level at least 50% greater in said biological sample than in said healthy tissue is
3 indicative of an epithelial cancer

1 51. The method of claim 46, wherein said epithelial cancer is
2 human breast cancer.

1 52. The method of claim 51, wherein said healthy tissue comprises
2 normal human mammary epithelial cells.

1 53. The method of claim 46, wherein abnormal expression of said
2 ESX gene is indicative of an unfavorable prognosis.

1 54. The method of claim 46, wherein said method further comprises
2 selecting an appropriate treatment regime.

1 55. The method of claim 46, wherein said detecting comprises
2 detecting an ESX nucleic acid.

1 56. The method of claim 55, wherein said detecting comprises a
2 hybridization assay.

1 57. The method of claim 46, wherein said detecting comprises
2 detecting an ESX polypeptide.

1 58. The method of claim 57, wherein said detecting comprises an
2 immunoassay.

1 59. The method of claim 58, wherein said ESX polypeptide is
2 detected using an antibody which specifically binds a polypeptide comprising at
3 least 10 contiguous amino acids from a polypeptide encoded by a nucleic acid as
A 4 set forth in ^{SEQ ID NO:1}~~SEQ ID NO:1~~.

1 60. The method of claim 59, wherein said nucleic acid is selected
2 from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:9, and
3 SEQ ID NO:11.

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1 61. A method of diagnosing an epithelial cancer in a patient, said
2 method comprising:
3 contacting a nucleic acid sample from the patient with a probe
4 which hybridizes selectively to a target polynucleotide sequence comprising a
5 sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ
6 ID NO:9, and SEQ ID NO:11 wherein the probe is contacted with the sample under
7 conditions in which the probe hybridizes selectively with the target polynucleotide
8 sequence to form a stable hybridization complex; and
9 detecting the formation of a hybridization complex.

1 62. The method of claim 61, wherein the nucleic acid sample is
2 from a patient with breast cancer.

1 63. The method of claim 61, wherein the nucleic acid sample is a
2 metaphase spread or a interphase nucleus.

1 64. A method of inhibiting growth or proliferation of neoplastic
2 cells, said method comprising administering to said cells an effective amount of an
3 agent that inhibits biological activity of an ESX transcription factor.

1 65. The method of claim 64, wherein said neoplastic cells comprise
2 a cancer in an organism.

1 66. The method of claim 64, wherein said agent inhibits expression
2 of said ESX transcription factor.

1 67. The method of claim 66, wherein said method comprises
2 transfecting cells of said mammal with vector expressing an antisense ESX nucleic
3 acid.

1 68. The method of claim 66, wherein said method comprises
2 administering to said organism a therapeutically effective dose of a composition
3 comprising an antisense ESX nucleic acid and a pharmacological excipient.

1 69. The method of claim 64, wherein said agent is an antibody that
2 specifically binds said ESX transcription factor.

1 70. The method of claim 64, wherein said agent is an inactive ESX
2 transcription factor mutein.

1 71. A transfected cell comprising a heterologous gene encoding an
2 ESX transcription factor.

1 72. The transfected cell of claim 71, wherein said cell comprises a
2 transgenic non-human animal.

1 73. The transgenic non-human animal of claim 72, wherein said
2 animal comprises a mutated ESX transcription factor gene and said animal is
3 deficient in ESX transcription factor activity.

1 74. The transgenic non-human animal of claim 73, wherein said
2 deficiency is a result of a reduced level of ESX mRNA compared to an unmutated
3 ESX gene in a similar milieu.

1 75. The transgenic non-human animal of claim 73, wherein said
2 deficiency is a result of said mutated gene encoding an ESX polypeptide having a
3 reduced level of biological activity compared to a wild-type ESX polypeptide.

1 76. The transgenic non-human animal of claim 73, wherein said
2 mutated gene comprises one or more mutations selected from the group consisting
3 of a missense mutation, a nonsense mutation, an insertion, or a deletion.

1 77. A method of determining whether a gene is regulated by an
2 ESX polypeptide, said method comprising the steps of:
3 a) contacting a nucleic acid comprising a 5' flanking region
4 of said gene with an ESX polypeptide to form a nucleic acid-protein complex;
5 b) treating said complex with a DNase under conditions
6 sufficient to digest said nucleic acid at hypersensitive regions;
7 c) separating said DNase-treated complex to obtain a
8 footprint pattern; and
9 d) determining whether said footprint pattern comprises a
10 hypersensitive band flanked by two protected regions, wherein said hypersensitive
11 band corresponding to a first guanine residue in a GGA sequence is indicative of
12 said gene being regulated by an ESX polypeptide.

1 78. A pharmacological composition comprising a pharmaceutically
2 acceptable carrier and a molecule selected from the group consisting of consisting
3 of a vector encoding an ESX nucleic acid or subsequence thereof, an ESX
4 polypeptide or subsequence thereof, and an anti-ESX antibody.

1 79. A kit for the detection of a *ESX* gene or polypeptide, said kit
2 comprising a container containing a molecule selected from the group consisting of
3 an ESX nucleic acid or subsequence thereof, an ESX polypeptide or subsequence
4 thereof, and an anti-ESX antibody.

1 80. A method of screening for a therapeutic lead compound, said
2 method comprising the steps of:
3 (i) providing a nucleic acid encoding a polypeptide of ESX
4 exon 4 or a polypeptide sequence of ESX exon 4;
5 (ii) contacting said nucleic acid or said polypeptide
6 sequence with said compound; and
7 (iii) detecting binding of said compound to said nucleic acid
8 or said polypeptide sequence.

1 81. A method of identifying potential therapeutic targets for drug
2 screening, said method comprising the steps of:
3 i) identifying a subsequence of the ESX gene or protein
4 necessary for ESX transactivational activity;
5 ii) performing a nucleic acid or protein database search to
6 identify other nucleic acids having significant sequence identity with said
7 subsequence whereby said subsequence is identified as a potential therapeutic
8 target for drug screening.

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